https://doi.org/10.29326/2304-196X-2024-13-3-248-254

Use of "ARRIAH-AviFluVac" vaccine in turkeys, geese and ducks

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ABSTRACT

"ARRIAH-AviFluVac" vaccine against H5 avian influenza was demonstrated to be effective for ducks, geese and turkeys both in the laboratory and production environment. When administered to ducks at 0.5; 1.0 and 1.5 cm^3, the vaccine provided 100%-effective protection of birds against the disease and death after challenge with the relevant high pathogenicity avian influenza virus of subtype H5N1, clade 2.3.4.4b. Singular 0.5–1.5 cm^3 inoculation induced formation of antibodies, which were detected in the hemagglutination inhibition test at the titres that ranged from 4.3 to 6.1 log_2. The vaccine facilitated 9–26-fold decrease in the virulent virus shedding by the ducks. Protection of turkeys vaccinated at the dose of 1.0 cm^3 was maintained at the level of 87.5% after challenge with high pathogenicity avian influenza virus of subtype H5N1, clade 2.3.4.4b. The vaccine induced formation of antibodies at the titres of 4.9 and 5.5 log_2 in turkeys after singular and double vaccination at the dose of 1.0 cm^3, respectively. It was demonstrated, that after double administration of 1.0 cm^3 of "ARRIAH-AviFluVac" vaccine, the post-vaccinal avian influenza antibody level exceeded 5.0 log_2 in 75.9–90.0% of the geese population. The most appropriate way of the vaccine use in turkeys, ducks and geese involves at least its double administration at the double commercial dose. Higher species resistance of ducks to the challenge with avian influenza virus of subtype H5, clade 2.3.4.4b as compared to turkeys was also demonstrated.

Keywords: high pathogenicity avian influenza, H5 avian influenza virus, inactivated vaccine, immunogenicity, ducks, geese, turkeys

Acknowledgements: The study was funded by the Federal Centre for Animal Health within the research topic "Veterinary Welfare".

For citation: Moroz N. V., Frolov S. V., Irza V. N., Scherbakova L. O., Kulakov V. Yu. Use of "ARRIAH-AviFluVac" vaccine in turkeys, geese and ducks. *Veterinary Science Today*. 2024; 13 (3): 248–254. https://doi.org/10.29326/2304-196X-2024-13-3-248-254

Conflict of interests: Irza V. N. is a member of the editorial board of the "Veterinary Science Today" journal, but was not involved into the decision making process related to this article publication. The manuscript has passed the review procedure accepted in the journal. The authors did not declare any other conflicts of interests.

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УДК 619:616.98:578.832.1:636.5:615.371:616-097.3

Применение вакцины «ВНИИЗЖ-АвиФлуВак» для индеек, гусей и уток

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РЕЗЮМЕ

В лабораторных и производственных условиях показано, что вакцина против гриппа птиц H5 «ВНИИЗЖ-АвиФлуВак» является эффективным препаратом для профилактики болезни у гусей, уток и индеек. Вакцина при введении уткам в дозах 0,5; 1,0 и 1,5 $см^3$ со 100%-й эффективностью защищала птиц от заболевания и гибели при заражении актуальным вирусом высокопатогенного гриппа птиц подтипа H5N1 генетической клады 2.3.4.4b. Однократная прививка в дозе от 0,5 до 1,5 $см^3$ вызывала образование антител, выявляемых в реакции торможения гемагглютинации, в титрах от 4,3 до 6,1 log_2. Вакцина способствовала снижению выделения вирулентного вируса утками в 9–26 раз. Протективная защита индеек, привитых в дозе 1,0 $см^3$, обеспечивалась на уровне 87,5% при заражении вирусом высокопатогенного гриппа птиц подтипа H5N1 клады 2.3.4.4b. Вакцина вызывала образование антител в титрах 4,9 и 5,5 log_2 у индеек при однократном и двукратном введении в дозе 1,0 $см^3$ соответственно. Установлено, что при двукратном применении препарата «ВНИИЗЖ-АвиФлуВак» в дозе 1,0 $см^3$ уровень поствакцинальных антител к вирусу гриппа птиц был выше 5,0 log_2 у 75,9–90,0% популяции гусей. Рациональным решением использования вакцины для индеек, уток и гусей является ее применение в двойной коммерческой дозе и как минимум двукратное введение. Также была установлена более высокая видовая устойчивость уток к заражению вирусом гриппа птиц подтипа H5 клады 2.3.4.4b по сравнению с индейками.

Ключевые слова: высокопатогенный грипп птиц, вирус гриппа птиц H5, инактивированная вакцина, иммуногенность, утки, гуси, индейки

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие».

Для цитирования: Мороз Н. В., Фролов С. В., Ирза В. Н., Щербакова Л. О., Кулаков В. Ю. Применение вакцины «ВНИИЗЖ-АвиФлуВак» для индеек, гусей и уток. *Ветеринария сегодня*. 2024; 13 (3): 248–254. https://doi.org/10.29326/2304-196X-2024-13-3-248-254

Конфликт интересов: Ирза В. Н. является членом редколлегии журнала «Ветеринария сегодня», но не имеет никакого отношения к решению опубликовать эту статью. Рукопись прошла принятую в журнале процедуру рецензирования. Об иных конфликтах интересов авторы не заявляли.

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INTRODUCTION

Avian influenza (AI) is a highly contagious viral disease that can affect several poultry species [1]. Over the past 10 years, high pathogenicity avian influenza (HPAI) H5Nx viruses of Eurasian genetic lineage Gs/Gd/96 clade 2.3.4.4 spread globally. In 2022–2023, an unprecedented HPAI panzootic occurred, which was caused by H5N1 virus of Eurasian genetic lineage 2.3.4.4b [2]. A huge number of the disease outbreaks in poultry and cases of the virus detection in wild and synanthropic birds of different species, as well as their mass mortality were reported in different countries. The virus was detected in terrestrial and marine mammals thus raising serious global concern due to pandemic threat (WOAH Situation Reports for Avian Influenza, 2021–2023; FAO Empres-i; WHO).

Starting from 2021, HPAI epizootics in the Russian Federation were also caused by H5N1 virus of Eurasian genetic lineage (clade) 2.3.4.4b [3].

Since the disease causes enormous damage to poultry farming and becomes enzootic in many countries, issues on the use of vaccination as an additional tool to contain the spread of infection and reduce unjustified losses were actively discussed at the international level in 2022–2023 [4, 5, 6]. The Terrestrial Animal Health Code (WOAH, Chapter 10.4) contains recommendations on poultry vaccination against HPAI and describes the conditions under which it can be used [7].

In most countries where AI vaccination is practiced, mainly whole-virion vaccines based on low-pathogenicity and genetically modified low-pathogenicity AI viruses obtained by reverse genetics and containing a hemagglutinin gene fragment of the relevant highly virulent H5 and H7 viruses are used.

Current inactivated AI vaccines are limited in effectiveness in *Anseriformes*, therefore, it is recommended to administer a double chicken dose, or to add a strong stimulant for effective immunity [8, 9, 10, 11]. It was also demonstrated that double dose of the bivalent vaccine protected ducks from the disease and death, but, however, the antibodies were formed at a low level (from 4 to 8 $\log_2$) and after challenge, the virus was isolated from 13% of vaccinated and 100% of unvaccinated birds. The inability of the challenge virus to induce the repeated production of antibodies in birds vaccinated with a closely related H5 strain is a convincing evidence of the lack of the virulent virus replication in the vaccinated ducks [12]. Most scientific articles show that the whole-virion vaccines are generally effective for ducks [9, 10, 13, 14, 15] and geese [10, 16], but these avian species react to vaccination in different ways [10, 17].

According to A. Kandeil et al., use of inactivated AI H5 vaccine induced development of an immune response in all avian species housed together on the backyards (ducks, geese and chickens), and after double vaccination the antibody titres reached 10 $\log_2$. The immune response was, however, different in different bird species. The vaccinated birds remained alive after challenge and shed less virus as compared to the unvaccinated ones. It should be noted that the unvaccinated ducks also did not get diseased and survived throughout the experiment. Moreover, the vaccinated ducks shed more virus than vaccinated birds of other species [10].

There are also many reports of positive results of genetically engineered vaccines used for HPAI prevention in poultry. Thus, according to E. Niqueux et al., simultaneous immunization with two recombinant Newcastle disease and fowlpox virus-vectored vaccines provided 12-week protection in Muscovy ducks [18]. Kim D. H. et al. also demonstrated that administration of genetically engineered Newcastle disease virus-vectored vaccine effectively protected Muscovy ducks from infection with the virulent HPAI H5 virus and decreased the virus shedding [19].

Most of the AI vaccine trials, both in the laboratory and in the field, are carried out in chickens and turkeys, because high mortality and excretion of a large amount of the virus into the environment are reported in them when infected. However, with the spread of AI in Asia, the disease epizootology changed, as indicated by the increased susceptibility of wild and exotic birds. Infection of domestic ducks and geese seriously affected the maintenance and spread of H5N1 AI [20].

In 2022, the Federal Centre for Animal Health (ARRIAH) registered "ARRIAH-AviFluVac" vaccine against AI (H5) based on the low-pathogenic H5 AI virus strain Yamal.

As part of the post registration process, studies were conducted to determine the vaccination dose and frequency of vaccination for various poultry species (turkeys, ducks and geese) in laboratory and field conditions, the results of which are demonstrated in this paper.

MATERIALS AND METHODS

Vaccine. "ARRIAH-AviFluVac" inactivated emulsion vaccine against avian influenza (H5) manufactured by the Federal Centre for Animal Health (batch 010122, release date 01.2022) was used in laboratory and field trails. The vaccine is prepared from the extraembryonic fluid of the chicken embryonated eggs infected with avian influenza virus (the source of H5N1 production strain Yamal is A/wildduck/YaNAO/956-21 isolate) inactivated with

aminoethylethanolamine supplemented with Montanide ISA 70 VG oil adjuvant (SEPPIC, France) at 30:70 w/w.

Poultry. Commercial poultry delivered from farms free from acute infectious avian diseases were used for laboratory tests: 20 day-old ducklings, 140 ducklings at the age of 21 days and 40 turkeys at the age of 10 days.

During the field trials, the vaccine was administered to commercial 1–28-day-old turkeys on one of the poultry farms in the Stavropol Krai and to the parent 30–60-day old goose flock in the Republic of Bashkortostan.

The experiment design at the animal keeping facilities. The day-old ducklings were divided into two groups of 10 birds in each. They were intramuscularly vaccinated at the doses of 0.25 and 0.5 cm^3, respectively; 28 days post immunization, the ducklings' blood sera were collected and tested for antibodies to AIV H5.

Twenty one-day-old ducklings were divided into 4 groups of 35 birds in each. The birds in group 1 were vaccinated with "ARRIAH-AviFluVac" vaccine at the dose of 0.5 cm^3; in group 2 – at the dose of 1.0 cm^3; in group 3 – at the dose of 1.5 cm^3. All ducklings were vaccinated intramuscularly into the thigh. The birds in group 4 were not vaccinated. After days 7, 14 and 21, blood samples were collected from the ducklings in each group to monitor seroconversion to AIV H5. Twenty eight days post vaccination, the birds in the experimental groups of 10 birds in each were challenged with the virulent influenza A H5 virus A/chicken/Stavropol/2077-6/21 H5N1 at the dose of 6.0 lg EID_{50} intramuscularly into the thigh at the volume of 0.5 cm^3. The challenged ducklings were subjected to 10-day clinical observation.

In 6 days post challenge, the oropharyngeal swabs were collected from the ducklings to identify the AIV genome by polymerase chain reaction (PCR).

Ten-day-old turkeys were divided into 4 groups of 10 birds in each. Poultry in the three experimental groups were intramuscularly vaccinated at the doses of 0.25; 0.5 and 1.0 cm^3, respectively. The turkeys in group 4 were not vaccinated. In 21 days post vaccination, blood was collected from the birds for testing for AIV H5 antibodies by hemagglutination inhibition test (HI). The turkeys were then challenged with A/chicken/Stavropol/2077/6/21 H5N1 virus at the dose of 6.0 lg EID_{50} intramuscularly into the thigh at the volume of 0.5 cm^3. The challenged birds were clinically observed for 10 days.

The experiment design in the field. An experimental group of 700 day-old turkeys was vaccinated at the dose of 0.2 cm^3. Then, at the age of 28 days the turkeys were divided into 2 groups of 350 birds in each and they were re-immunized at the doses of 0.5 and 1.0 cm^3, respectively.

Two groups of 30 geese were formed. The birds in group 1 were intramuscularly vaccinated once at the age of 30 days at the dose of 1.0 cm^3; the birds in group 2 were also intramuscularly vaccinated twice at the age of 30 and 60 days at the dose of 1.0 cm^3.

All experiments on birds were conducted in strict compliance with the interstate standards for animal handling and housing adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Serological tests for AIV H5 antibodies were carried out by HI test using the ARRIAH-manufactured HI test-kit for detection of antibodies against H5 avian influenza virus. HI results were expressed in log_2, and an antibody titre equal to or higher than 5 log_2 was considered to be the minimum protective titre according to the recommendations of the World Organization for Animal Health [21].

Challenge. Resistance of vaccinated poultry to the challenge with A/chicken/Stavropol/2077/6/21 H5N1 AIV at the dose of 6.0 lg EID_{50} was justified by the absence of mortality and disease clinical signs (depression, respiratory and nervous disorders) in the poultry.

Molecular genetic tests. Detection of AIV genome in biomaterial samples and determination of the amplification cycle threshold were carried out in accordance with the "Methodological recommendations for detection of Type A avian influenza virus RNA by real-time RT-PCR"[1].

Oropharengeal swabs were collected with sterile tupfers. The samples were collected from all ducklings in the relative groups in 6 days post challenge. Amplification cycle thresholds (*Ct*) were determined in the control group (Ct_0) and in the groups of the vaccinated birds (Ct_i). The reaction was considered positive (AIV H5 genome is present) if $0 < Ct < 37$ [21]. Next, a comparison with the control was performed and the difference of the compared values was calculated ($d_i = Ct_i - Ct_0$). In addition, the average estimates of the difference (*D*) and standard errors of measurement of the averages (± *m*) were calculated from the group samples (*d*).

The immunogenicity of "ARRIAH-AviFluVac" vaccine for ducks was determined in laboratory experiments by the results of the resistance to challenge, by the titres of serum antibodies and by PCR-indicated virus shedding.

The immunogenicity of "ARRIAH-AviFluVac" vaccine for turkeys was determined in laboratory and field experiments based on the results of the resistance to challenge and serum antibody titres.

The immunogenicity of "ARRIAH-AviFluVac" vaccine for geese was determined in the field experiments by serum antibody titres.

Statistical processing of the resulted data included determination of mean values, error of mean, statistical significance of the differences between the experimental groups of animals along with the indication of the statistical criterion values, number of degrees of freedom and prediction error probability (*p*).

RESULTS AND DISCUSSION

Table 1 demonstrates the HI results of the sera of ducklings of different ages tested for the antibodies to AIV H5 after vaccination.

It was found that day-old ducklings vaccinated at the dose of 0.25 cm^3 demonstrated AIV H5 antibodies in low nonuniform titres ($p > 0.05$). At the same time, the AIV H5 antibody titres in the group of ducklings vaccinated at the dose of 0.5 cm^3 reached 4.4 log_2 with good uniformity in 28 days ($p \leq 0.001$).

The vaccinated 21-day-old ducklings demonstrated AIV H5 antibodies in comparable titres regardless of the vaccine dose (0.5; 1.0 or 1.5 cm^3). Seroconversion

[1] Andriyasov A. V., Andreychuk D. B., Chvala I. A. Methodological recommendations for detection of Type A avian influenza virus RNA by real-time RT-PCR: No. 45-16. Vladimir: ARRIAH; 2016. 13 p. (in Russ.)

Table 1
Results of duckling serum tests for AIV H5 antibodies using HI test

Age of birds at the time of vaccination, day	Vaccination dose, cm^3	Antibody titres (log_2) at different time points post vaccination			
		Day 7	Day 14	Day 21	Day 28
1	0.25	n/t	n/t	n/t	1.3 ± 0.6
	0.5	n/t	n/t	n/t	4.4 ± 0.3
21	0.5	0.9 ± 0.6*	0.9 ± 0.6 (0)	4.3 ± 0.5 (4,5)	4.4 ± 0.6
	1.0	3.1 ± 0.6	3.1 ± 0.6 (4.0)	5.0 ± 0.4 (5.0)	5.1 ± 0.4
	1.5	2.1 ± 0.8 (1.5)	2.1 ± 0.8 (1.5)	6.1 ± 0.5 (6.0)	6.2 ± 0.6

* mean HI antibody titre and error, median (Me) is in brackets; n/t – not tested.

was observed in groups of immunized ducklings 7 days later with antibody titres reaching the maximum levels in 28 days post vaccination.

Statistical processing of the primary antibody titres obtained 28 days post vaccination in the experimental groups was carried out by one-way analysis of variance in Excel. It was found that the F-test statistics was below F critical value ($F_{test}3.0 < F_{critical}3.4$), that is, the mean antibody titres for the three groups did not differ.

To determine the protective properties of the vaccine against the H5 HPAI pathogen, the vaccinated ducks were challenged with A/chicken/Stavropol/2077-6/21 H5N1 virus. Table 2 demonstrates the results of the experiment.

It was found that the vaccinated ducklings in all experimental groups did not get diseased within 10 days after challenge with the high pathogenicity AI H5N1 virus. In the group of the unvaccinated birds, six birds demonstrated the disease signs and one bird died.

In order to establish the immunity level in the vaccinated ducks, the studies were performed to detect the virulent virus shedding. This stage was targeted at the detection of AIV H5 genome in the oropharyngeal secretions of the birds in 6 days after the challenge.

It was revealed that AIV H5 genome was present in all tested samples. However, the initial concentration of the viral material in the oropharyngeal excretions of the vaccinated birds was significantly lower as compared to the controls. Thus, amplification cycle thresholds (*Ct*) in the control group ranged from 20.93 to 25.47 (average – 23.83), in the group of ducklings vaccinated at the dose of 0.5 cm^3 – from 24.49 to 29.46 (average – 26.84), in the group of ducklings vaccinated at the dose of 1.0 cm^3 – from 23.08 to 30.29 (average – 27.5), in the group of ducklings vaccinated at the dose of 1.5 cm^3 – from 26.12 to 31.41 (average – 28.32). Considering that one amplification cycle approximately involves doubling of the amount of the target product, the initial concentration of the viral material in the test sample (%) as compared to the control can be described as $J = (1/2^D) \times 100$. Hence, by the groups of the vaccinated birds, the target estimates were (%): $J_I = 10.8$; $J_{II} = 6.9$ and $J_{III} = 3.9$, i.e. the ducks vaccinated at the dose of 0.5 cm^3 shed the virus in 9-fold lesser amount, at the dose of 1.0 cm^3 – in 14-fold lesser amount, and at the dose of 1.5 cm^3 – in 26-fold lesser amount as compared to the unvaccinated birds.

The vaccination of ducklings, therefore, contributed to the decrease of the virus shedding by the vaccinated birds compared with the unvaccinated ones, and the higher the vaccine dose, the more significant the decrease was.

To determine the protective properties of the vaccine against the AIV H5 pathogen, the vaccinated turkeys were infected with A/chicken/Stavropol/2077-6/21 H5N1 virus in the laboratory.

The data presented in Table 3 demonstrate that the resistance of the vaccinated turkeys to infection varied in different groups. Thus, turkeys vaccinated at the dose of 1.0 cm^3 (87.5%) had the highest resistance, and the turkeys vaccinated at the dose of 0.25 cm^3 (28.6%) had the lowest resistance. The unvaccinated turkeys died in 3 days post infection.

Postvaccinal humoral immune response was determined in turkeys before challenge. It was found that in the group of birds vaccinated at the dose of 0.25 cm^3, the average antibody titre was 3.3 ± 0.6 log_2, in the group vaccinated at the dose of 0.5 cm^3 – 4.0 ± 0.6 log_2, and in the group vaccinated at the dose of 1.0 cm^3 – 4.9 ± 0.4 log_2.

Thus, single vaccination of turkeys at the dose of 1.0 cm^3 induced the most intense immune response to AIV H5, which was expressed by high protection level (87.5% of the birds did not get diseased) and high antibody titres.

In addition to the laboratory tests, field trials of the vaccine were carried out in commercial turkeys in the Stavropol Krai.

The turkey immunity level to AIV H5 was assessed by HI antibody titres in 35 days after the second vaccination.

Table 2
Resistance of vaccinated ducklings to challenge with subtype H5N1 influenza A virus

Observation period, days	Groups by the inoculation dose, cm^3			
	0.5	1.0	1.5	Control
1	0/10*	0/10	0/10	0/10
2	0/10	0/10	0/10	0/10
3	0/10	0/10	0/10	0/10
4	0/10	0/10	0/10	0/10
5	0/10	0/10	0/10	2/10
6	0/10	0/10	0/10	5/9 (1 dead)
7	0/10	0/10	0/10	5/9
8	0/10	0/10	0/10	5/9
9	0/10	0/10	0/10	5/9
10	0/10	0/10	0/10	5/9

* ratio between the diseased ducklings to the total number of duckling in the group.

Table 3
Resistance of vaccinated turkeys to challenge with subtype H5N1 influenza A virus

Observation period, days	Groups according to the vaccination dose, cm^3			
	0.25	0.5	1.0	Control
1	0/7*	0/9	0/8	0/8
2	0/7	0/9	0/8	2/8
3	0/7	0/9	0/8	6/6
4	1/7	0/9	0/8	–
5	2/6	1/9	0/8	–
6	1/4	2/8	0/8	–
7	1/3	1/6	1/8	-
8	0/2	0/5	0/7	–
9	0/2	1/4	0/7	–
10	0/2	0/4	0/7	–
Protection level, %	28.6 (2/7)**	44.4 (4/9)	87.5 (7/8)	0 (0/8)

* ratio between the dead birds to the total number of birds in the group;
** ratio between the survived birds to the total number of birds in the group.

It was found that the average antibody titres in the group of turkeys vaccinated twice at the dose of 1.0 cm^3 were 5.5 ± 0.2 log_2, and in the group vaccinated at the dose of 0.5 cm^3 – 3.5 ± 0.3 log_2. Statistical results differed with high degree of confidence (99.9%).

Based on the results of the laboratory tests and field trials of "ARRIAH-AviFluVac" vaccine in turkeys, the optimal parameters of its administration were established, i.e.: dose – 1.0 cm^3, frequency – at least double vaccination.

Studies were also conducted to examine the vaccine immunogenicity in commercial geese in the Republic of Bashkortostan.

The data in Table 4 demonstrate that after single immunization, the vaccine induced formation of AIV H5 antibodies in 48.5% of the goose population at the titre of 3.7 log_2 one month after vaccination; and in two months after immunization, the number of birds with protective antibody titres was only 23.3% and the average group titre was 1.9 log_2.

As the study results demonstrated, the minimum protective AIV H5 antibody titre (≥ 5 log_2) after double vaccination was reported in 22–27 out of 30 birds for 10 months, i.e. the protection coverage was at the level of 75.9–90.0% of the population. During this period, the average antibody titres were also at a high level and ranged from 6.3 to 6.8 log_2.

CONCLUSION

Single vaccination of ducklings with "ARRIAH-AviFluVac" vaccine at the dose of 0.5–1.5 cm^3 induced HI-detected formation of antibodies in titres from 4.3 to 6.1 log_2, which is consistent with the data reported by D. Middleton [12]. It was demonstrated that the ducklings were resistant to infection with the AIV H5N1 28 days after single administration of "ARRIAH-AviFluVac" vaccine at doses of 0.5, 1.0 and 1.5 cm^3. It is also worth mentioning that the initial concentration of the viral material in the oropharyngeal excreta of the vaccinated birds was 9–26 times lower as compared to the unvaccinated birds.

Thus, the vaccine has a high antigenicity sufficient for the formation of immunity in ducklings when administered at the doses from 0.5 to 1.5 cm^3. It was also demonstrated that inoculation of day-old ducklings at the dose of 0.25 cm^3 was insufficient to protect the birds.

The unvaccinated ducklings were less susceptible to the infection with the virulent H5 virus of clade 2.3.4.4b as compared to turkeys, and 60% of the birds were diseased, which is consistent with the data on low sensitivity of unvaccinated ducks when infected with the H5 virus of clade 2.2.1.2 H5N1 reported by A. Kandeil et al. [10]. This is indicative of the capacity of the virus carrier ducks to maintain the pathogen reservoir.

The laboratory study results demonstrated that the optimal inoculation dose of "ARRIAH-AviFluVac" vaccine for turkeys was 1.0 cm^3, when the vaccinated birds were protected from the infection by 87.5% and antibodies were formed at the highest titres (4.9 log_2).

In the field trials, the vaccine effectiveness was also demonstrated when used twice at the dose of 1.0 cm^3, when it was possible to gain high antibody titres (5.5 log_2) in commercial turkeys. Based on the data resulted from the laboratory and field trials, it was found that the dose of 1.0 cm^3 is optimal for use in turkeys, and the frequency of vaccination should be at least double vaccination.

In the field trials, two schemes of "ARRIAH-AviFluVac" administration were tested in geese, and it was found that when administered twice at the dose of 1.0 cm^3 the vaccine provided 10-month immunity in 75.9–90.0% of the poultry population.

The data obtained are consistent with the conclusions of a number of scientists [8, 9, 10, 11] and indicate that double administration of "ARRIAH-AviFluVac" vaccine against AI H5 at a double commercial dose and at least twice is reasonable for large and domestic waterfowl species with subsequent control of immunity level and revaccination by indications.

Table 4
Results of goose serum tests for post-vaccinal AIV H5 antibodies

Vaccination frequency	Antibody titres (log_2) at different time points after vaccination				
	day of vaccination	1 month	2 months	7 months	10 months
Single	n/d	3.7 ± 0.6 (16/33*; 48.5%)	1.9 ± 0.4 (7/30; 23.3%)	n/d	n/d
Double	0.8 ± 0.3	6.3 ± 0.5 (25/30; 83.3%)	n/d	6.5 ± 0.5 (22/29; 75.9%)	6.8 ± 0.3 (27/30; 90.0%)

* seroconversion is expressed as the ratio between the number of birds demonstrating HI antibody titre above 5 log_2 and total number of tested birds (%);
n/d – no data.

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Received 15.04.2024
Revised 03.06.2024
Accepted 08.07.2024

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